CAB INTERNATIONAL
MYCOLOGICAL INSTITUTE
LIBRARY
4 FEB 1992
FULTON, H.R.

G-658

# THE FUNGICIDAL ACTION OF ULTRA-VIOLET RADIATION

1929

BY

HARRY R. FULTON AND W. W. COBLENTZ

(Contribution from Bureau of Plant Industry)

*Reprinted from* JOURNAL OF AGRICULTURAL RESEARCH
Vol. 38, No. 3 : : : Washington, D. C., February 1, 1929

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

U. S. GOVERNMENT PRINTING OFFICE : 1929

## JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

**FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE**

E. W. ALLEN, CHAIRMAN
*Chief, Office of Experiment Stations*

C. L. SHEAR
*Senior Pathologist in Charge, Mycology and Disease Survey*

A. C. BAKER
*Senior Entomologist in Charge, Tropical and Subtropical Plant Insect Investigations*

**FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES**

W. J. MORSE
*Director, Maine Agricultural Experiment Station*

M. J. FUNCHESS
*Director, Alabama Experiment Station*

G. A. DEAN
*Head, Department of Entomology, Kansas Agricultural Experiment Station*

**EDITORIAL SUPERVISION**

M. C. MERRILL
*Chief of Publications, United States Department of Agriculture*


All correspondence regarding articles from State experiment stations should be addressed to W. J. Morse, Director, Agricultural Experiment Station, Orono, Me.


Published on the first and fifteenth of each month. This volume will consist of twelve numbers and the Contents and Index.

*Subscription price:* Domestic, $4.00 a year (two volumes)
Single numbers, 20 cents
Foreign, $5.00 a year (two volumes)
Single numbers, 25 cents

If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer, and they will be supplied practically at cost. Single copies of separates may be obtained free of charge from the Office of Information, United States Department of Agriculture, until the supply is exhausted.

Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

# THE FUNGICIDAL ACTION OF ULTRA-VIOLET RADIATION[1]

By HARRY R. FULTON, *Senior Pathologist, Office of Fruit Diseases, Bureau of Plant Industry, United States Department of Agriculture*, and W. W. COBLENTZ, *Physicist, Bureau of Standards, United States Department of Commerce*

## INTRODUCTION

The bactericidal and fungicidal action of ultra-violet rays has been well recognized since the pioneer investigations of Downes and Blount[2] and of Marshall Ward.[3]

With the development of the quartz-inclosed mercury arc as a powerful source of ultra-violet radiation a number of practical applications have been made. Among these is the sterilization of various substances, for the most part liquids, through the killing action of the rays on bacteria and fungi.

The problem here investigated in a preliminary way is the surface disinfection of citrus fruits, which are opaque to the rays, with a view to reducing or retarding the development of decay, especially that caused by *Penicillium digitatum*.

## EXPERIMENTAL PROCEDURE

The source of the ultra-violet radiation used in this investigation was a 110-volt quartz burner having a mercury cathode and a tungsten anode, normally operated on 320 watts (80 volts, 4 amperes). A water-cooled stage, consisting of a copper plate mounted in contact with a coil of thin-walled (0.1 mm.) copper tubing through which cold water was circulated, was used underneath exposed Petri dishes, to prevent overheating during prolonged irradiation. A detailed description of this apparatus and of the spectral-energy distribution of the mercury arc has been given in a previous paper.[4]

In making tests in Petri dishes the usual procedure developed after preliminary tests with other methods was to pour uninoculated nutrient agar, allow it to harden, and then apply the fungus spores to the surface as uniformly as possible either by barely flooding the surface with a few drops of a water suspension of the spores or by atomizing. In the latter case it was found advantageous to direct the nozzle of the atomizer upward and to hold the Petri dish in an inverted position some 30 cm. away so as to avoid the larger droplets. The exposures were made with the glass covers removed. Frequently a portion of

---

[1] Received for publication Oct. 1, 1928; issued February, 1929. This investigation was conducted as a cooperative project by the Bureau of Plant Industry of the U. S. Department of Agriculture and the Bureau of Standards of the U. S. Department of Commerce.

[2] DOWNES, A., and BLUNT, T. P. RESEARCHES ON THE EFFECT OF LIGHT UPON BACTERIA AND OTHER ORGANISMS. Roy. Soc. [London], Proc. (1877) 26:488-500. 1878.

——— and BLUNT, T. P. ON THE INFLUENCE OF LIGHT UPON PROTOPLASM. Roy. Soc. [London], Proc. 28:199-212, illus. 1878.

[3] WARD, H. M. FURTHER EXPERIMENTS ON THE ACTION OF LIGHT ON BACILLUS ANTHRACIS. Roy. Soc. [London], Proc. 53:34. 1893.

——— THE ACTION OF LIGHT ON BACTERIA.—III. Roy. Soc. [London], Phil. Trans. (B, 1894) 185:961-986, illus. 1895.

[4] COBLENTZ, W. W., and FULTON, H. R. A RADIOMETRIC INVESTIGATION OF THE GERMICIDAL ACTION OF ULTRA-VIOLET RADIATION. U. S. Dept. Com., Bur. Standards Sci. Paper 495, p. 641-680, illus. 1924.

the plate was screened with aluminum to serve as a check, or entirely unexposed plates inoculated similarly to the test plates were held as controls. The plates after exposure were kept at room temperature for several days until proper growth records could be made.

The spores of *Penicillium digitatum* and of *P. italicum* were usually taken from vigorously sporulating growth on recently rotted citrus fruits. No special effort was made to overcome the tendency of these spores to clump when mixed with water, because it was deemed important to conduct the tests with spore material such as might be found under practical working conditions in the citrus-packing industry. The spores of other fungi used in these tests were taken from freshly sporulating pure cultures.

## PRELIMINARY TESTS WITH PENICILLIUM SPORES

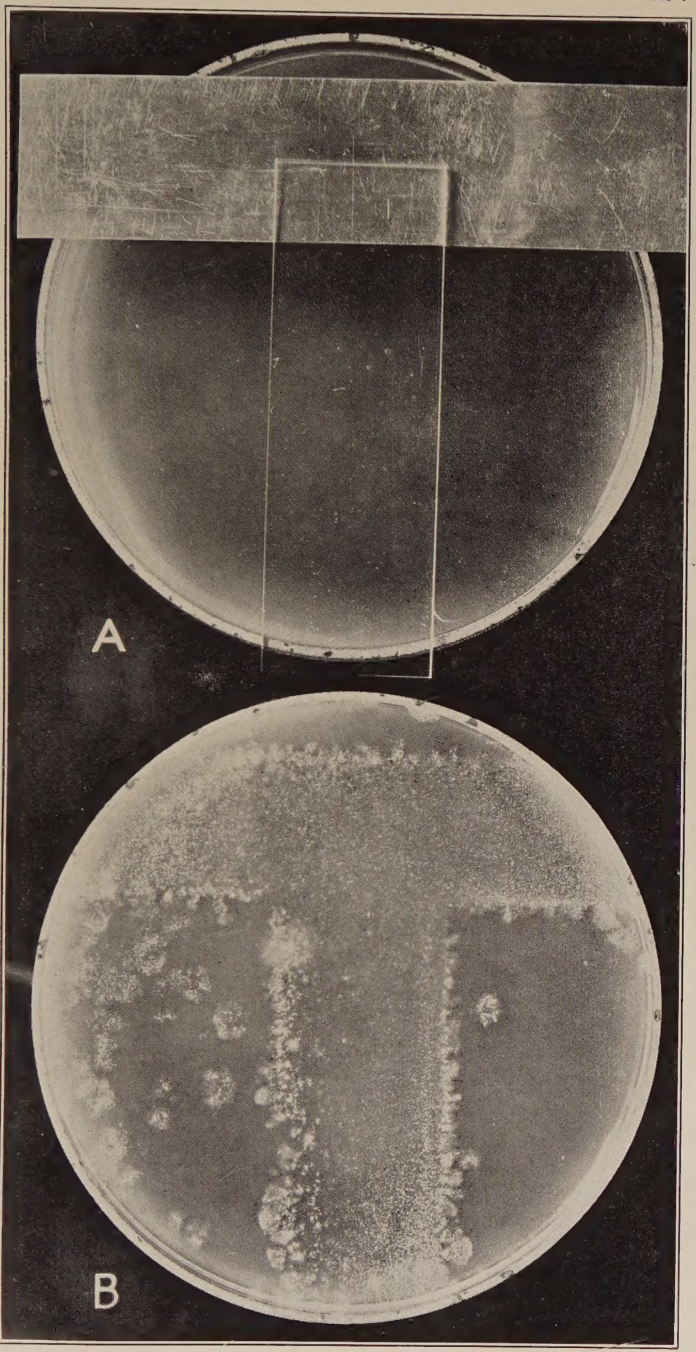
Shake cultures of *Penicillium digitatum* spores were made in cornmeal agar and poured to uniform depth in Petri dishes five hours before irradiation. Exposures at 7 inches from the lamp for 1, 3, 8, and 16 minutes resulted in reducing the after development of colonies to 25, 10, 5, and 2 per cent, respectively, as compared with 100 per cent developing in the check areas on the same plates screened with a 1-inch wide aluminum strip placed across the dish. (Pl. 1, A.) Microscopic examination showed that most of the growth in the irradiated portions came from clusters of spores where the outer ones doubtless had a protective screening effect upon the central ones of a group. Many of the surviving colonies were at the very margin of the Petri dish, where there may have been a protective influence on account of greater depth of agar or partial shading from the sides of the dish. The longer exposures developed a considerable heating effect, which may have had some disturbing influence. The area under the narrow aluminum strip, especially at its margins, however, must have been practically as warm as the adjacent portions of the irradiated area. The sharp killing effect at the boundary indicated that the heat developed was not sufficient, at least in itself, to kill. The growth under the aluminum strips was in every way comparable to that on entirely unexposed check plates of the same sowing. (Pl. 1, B.)

At the edges of thickly sown areas and where only a few colonies survive in the irradiated areas, the growth is very vigorous. Probably this is due mainly to freedom from crowding. It is also possible that the less than lethal dosages have a stimulating effect, such as has been noted by other investigators.[5]

A comparison was made of the effects of 5-minute exposures on poured plates of *Penicillium digitatum* spores distributed in cornmeal agar poured 24 hours, 6 hours, and 1 hour before irradiation. The sowing was at the rate of about 100 spores per square centimeter. The percentages of survival were estimated as 2, 5, and 10 per cent, respectively. This is taken to mean that growing mycelium is more readily killed than ungerminated spores.

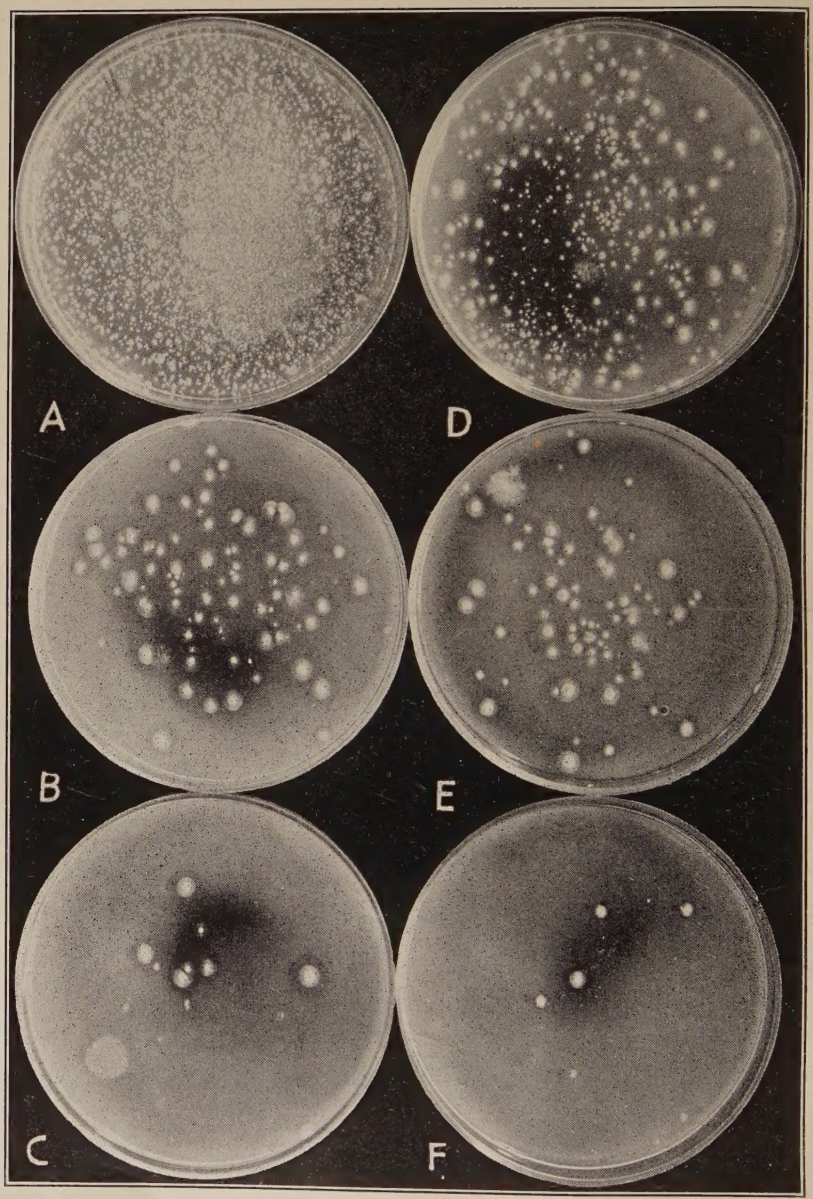
Since the agar itself has a protective influence, because of absorption of the rays, surface-smear sowings of *Penicillium digitatum* at

[5] DUFRÉNOY, J. ACTION DES RADIATIONS ULTRA-VIOLETTES SUR LES ZOOSPORES DE BLEPHAROSPORA CAMBIVORA PETRI ET DE PHYTOPHTHORA OMNIVORA PARASITICA. Rev. Path. Vég. et Ent. Agr. 12: [270]–272, illus. 1925.




A.—Petri dish showing the arrangement of an aluminum strip and a glass microscope slide used as screens during exposure to ultra-violet radiation

B.—Petri dish showing the practically equal effect of aluminum and glass screens in protecting *Penicillium digitatum* spores that were very thickly atomized over the nutrient agar on the plate. Exposure was for 3 minutes at 13 inches from the ultra-violet lamp, and the photograph was made 3 days later. Colonies at the edge of the protected areas and the scattered survivors in the irradiated areas are less crowded and show more extensive growth




AGAR PLATES ATOMIZED WITH A HEAVY SPORE SUSPENSION OF PENICILLIUM DIGITATUM

A.—Unexposed check.

B.—Irradiated discontinuously for a total of 15 seconds in three 5-second exposures with 5-second intervals.

C.—Irradiated discontinuously for a total of 45 seconds in nine 5-second exposures with 5-second intervals.

D, E, and F.—Irradiated 5, 15, and 45 seconds, respectively, with continuous exposures.

All plates were exposed 6 inches from the ultra-violet lamp and photographed three days after irradiation. Compare B and C with E and F.

the rate of about 20 spores per square centimeter were made on corn-meal agar plates 24 hours, 6 hours, and 1 hour before 5-minute exposure to the ultra-violet rays. The result was a complete killing of all spores. A comparison with the results outlined in the two preceding paragraphs indicates that the full intensity of the rays under the conditions indicated is sufficient to kill all spores of this species, whether in clumps or not, if the exposure is sufficiently prolonged.

In parallel tests with *Phomopsis citri*, the poured-plate sowings averaged about 50 spores per square centimeter and the surface smears about 20 spores per square centimeter. An irradiation of five minutes completely killed all spores for both methods of testing. Two obvious differences between Phomopsis and Penicillium spores might be factors in the more ready killing of the former. The walls of Phomopsis spores are thin and not highly colored, and these spores do not tend to cling together in clumps.

In another test, corn-meal agar plates were very heavily inoculated by flooding the surface with *Penicillium digitatum* spores. It was estimated from growth on check plates that there were 1,000,000 spores per plate. Exposures were made at 7 inches distance from the lamp for one-half, one, three, and five minutes. Three days later there was growth of 60, 82, 32, and 0 colonies, respectively, per plate. Similar plates exposed at a distance of 13 inches from the lamp and for the same periods of time showed later development of 85, 60, 35, and 8 colonies, respectively. The best survival shown in this series is less than 1 in 10,000 of the spores originally sown. The only complete kill obtained was with an exposure ten times as long and an intensity almost four times as great as was required to kill 9,999 out of 10,000 of the spores. In other words, the killing of absolutely all *P. digitatum* spores requires an extremely large amount of energy as compared with that required to kill 99.99 per cent of the spores. The main reason for the erratic survival of a relatively small number of spores after exposure for excessively long periods of irradiation is presumed to be the clumping of the Penicillium spores in such a way as to shade some from the rays.

## PRELIMINARY TESTS WITH VARIOUS TYPES OF SPORES

A test was made on a number of miscellaneous fungi that happened to be readily available to determine differences in resistance to the abiotic effect of the ultra-violet rays. Water suspensions of spores taken from pure cultures were applied as counted drops on the surface of hardened corn-meal agar in Petri dishes, and the drops were spread over the surface as evenly as possible. Exposures of the uncovered dishes to the ultra-violet rays were made at a distance of 6 inches and for 1-minute and 4-minute periods. Unexposed plates were held as checks. Records were made after six or eight days. The results are given in Table 1.

Since killing was complete in one minute for most of these spores, a series of exposures shorter than one minute would have been required to bring out more completely the differences in resistivity. At the time of the above-mentioned test, plates of Hainesia spores were exposed for 15 seconds and for 30 seconds. These showed 4 surviving in the first instance and 1 in the second, out of an estimated sowing of 5,000 spores.

TABLE 1.—*Killing effect of ultra-violet radiation on various kinds of fungus spores*

| Organism tested | Total number of colonies | Colonies surviving after exposure for— | | | |
|---|---|---|---|---|---|
| | | One minute | | Four minutes | |
| | | Number | Per cent | Number | Per cent |
| Diplodia sp. (from lime fruit) | 17 | 9 | 52.9 | 7 | 41.2 |
| Helminthosporium sp. (from cranberry) | 55 | 23 | 41.8 | 26 | 47.3 |
| Alternaria sp. (from cranberry) | 81 | 28 | 34.6 | 14 | 17.3 |
| Cladosporium sp. (from cranberry) | 400 | 62 | 15.5 | 18 | 4.5 |
| Aspergillus niger | 1,500 | 200 | 13.3 | 8 | .5 |
| Alternaria citri | 600 | 60 | 10.0 | 13 | 2.2 |
| Pestalozzia sp. (from cranberry) | 1,000 | 20 | 2.0 | 22 | 2.2 |
| Penicillium italicum | 2,000 | 14 | .7 | 2 | .1 |
| Chaetomella sp. (from cranberry) | 600 | 2 | .3 | 0 | 0 |
| Hainesia sp | 5,000 | 1 | Trace. | 0 | 0 |
| Colletotrichum gloesporioides | 10,000 | 1 | Trace. | 0 | 0 |
| Ceuthospora lunata | 200 | 0 | 0 | 0 | 0 |
| Rhizopus nigricans | 1,000 | 0 | 0 | 0 | 0 |
| Penicillium digitatum | 6,000 | 0 | 0 | 0 | 0 |
| Penicillium expansum | 1,200 | 0 | 0 | 0 | 0 |
| Phomopsis citri | 800 | 0 | 0 | 0 | 0 |
| Fusarium sp. (from orange fruit) | 1,200 | 0 | 0 | 0 | 0 |
| Botrytis sp. (from apple) | 20 | 0 | 0 | 0 | 0 |
| Glomerella rufomaculans | 800 | 0 | 0 | 0 | 0 |
| Phytophthora sp. (from orange fruit) | 4 | 0 | 0 | 0 | 0 |
| Monilia fructigena | 200 | 0 | 0 | 0 | 0 |
| Gloeosporium limetticolum | 10 | 0 | 0 | 0 | 0 |
| Anthostomella destruens | 104 | 0 | 0 | 0 | 0 |
| Guignardia sp. (from cranberry) | 600 | 0 | 0 | 0 | 0 |
| Melanconium sp. (from grape) | 800 | 0 | 0 | 0 | 0 |
| Acanthorhynchus vaccinii | 900 | 0 | 0 | 0 | 0 |
| Gloeosporium minus | 1,000 | 0 | 0 | 0 | 0 |

It is to be noted that the more resistant fungi, as a group, are the ones having dark spore walls. Of the two common rots of citrus, *Penicillium italicum* seemed somewhat more resistant than *P. digitatum*, but it did not show an extraordinary percentage of survival.

The considerable difference in resistivity of different kinds of fungus spores suggests the possibility of using ultra-violet radiation in certain special cases to free mixed inoculum from undesirable contaminating organisms, in case these are more easily killed than the ones desired. Nonsporulating bacteria, as a rule, are more quickly killed than most fungus spores, and quick-germinating fungus spores might be killed by a dosage that would not destroy ungerminated types. Another laboratory use might be the surface disinfection of thin leaves or other delicate plant parts before culturing for organisms growing inside the tissues.

## EFFECT OF INTERMITTENT EXPOSURES

The practical use of ultra-violet radiation for the surface disinfection of citrus fruits would involve intermittent exposure of any particular portion of the surface as the fruits are slowly revolved in passing on carriers under a battery of lamps. It is important to determine whether or not a series of short exposures would produce approximately the same killing effect as a single prolonged exposure of equal total duration. Corn-meal agar plates were atomized over the surface with a suspension of *Penicillium digitatum* spores and exposed at a distance of 6 inches from the lamp. A parallel series is shown for *Bacillus coli*, beef agar being used in this case. Observations were made three days after setting up. The results are given in Table 2.

TABLE 2.—*Abiotic action of ultra-violet rays on Penicillium digitatum and on Bacillus coli, showing comparative effect of continuous and discontinuous exposure*

| Total duration of exposure | Method of exposure | Colonies surviving (per 1,000) | |
|---|---|---|---|
| | | Penicillium digitatum | Bacillus coli |
| 5 seconds | 1 exposure of 5 seconds, continuous | 93.3 | 2.7 |
| 10 seconds | 1 exposure of 10 seconds, continuous | 46.7 | .8 |
| | 2 exposures of 5 seconds each, with 1 rest of 5 seconds | 73.3 | 1.3 |
| 15 seconds | 1 exposure of 15 seconds, continuous | 29.3 | 1.3 |
| | 3 exposures of 5 seconds each, with 2 rests of 5 seconds | 32.0 | .7 |
| 20 seconds | 1 exposure of 20 seconds, continuous | 14.6 | .2 |
| | 4 exposures of 5 seconds each, with 3 rests of 5 seconds | 20.7 | .2 |
| 30 seconds | 1 exposure of 30 seconds, continuous | 10.3 | .2 |
| | 6 exposures of 5 seconds each, with 5 rests of 5 seconds | 14.0 | .4 |
| 45 seconds | 1 exposure of 45 seconds, continuous | 2.3 | 0 |
| | 9 exposures of 5 seconds each, with 8 rests of 5 seconds | 3.3 | 0 |
| | Average estimates for unexposed check plates | 3,000 | 9,000 |
| 30 seconds | 1 exposure of 30 seconds, continuous | 3.3 | 8.3 |
| | 3 exposures of 10 seconds each, with 2 rests of 10 seconds | 8.3 | 10 |
| | Average estimates for unexposed check plates | 1,200 | 6,000 |

While the continuous exposures gave a somewhat better kill than discontinuous ones of the same total duration, the differences do not seem to be great enough to be of importance in practical applications. (Pl. 2.) Wherever more than two short exposures were used the measure of the differences was less than 1 per cent of the organisms originally sown on the plates.

In a previous report[6] the writers have presented other experimental data substantiating this finding, based on experiments in which *Bacillus coli* was used as the test organism and in which the intensity of the radiation was greatly reduced so as to prolong the time required to kill, thus providing for a more accurate determination of the effect.

An attempt has been made to use the data presented in Table 2 for roughly evaluating what may be termed the killing effect upon *Penicillium digitatum* of each successively added 5-second period for both the continuous and the discontinuous applications. This has been done by estimating the percentage killed during each 5-minute period, each percentage being based on the number of spores living at the beginning of the particular period. Table 3 gives the results of these computations.

TABLE 3.—*Estimates of the killing effect upon Penicillium digitatum of successively added 5-second periods of exposure to ultra-violet rays continuously and discontinuously applied*

| Periods | Per cent killed during each period of— | |
|---|---|---|
| | Continuous exposure | Discontinuous exposure |
| First 5 seconds of exposure | 91 | ---------- |
| Second 5 seconds of exposure | 50 | 21 |
| Third 5 seconds of exposure | 37 | 56 |
| Fourth 5 seconds of exposure | 50 | 35 |
| Fifth and sixth 5 seconds of exposure, average | 14 | 16 |
| Seventh, eighth, and ninth 5 seconds of exposure, average | 26 | 25 |

[6] COBLENTZ, W. W., and FULTON, H. R. Op. cit.

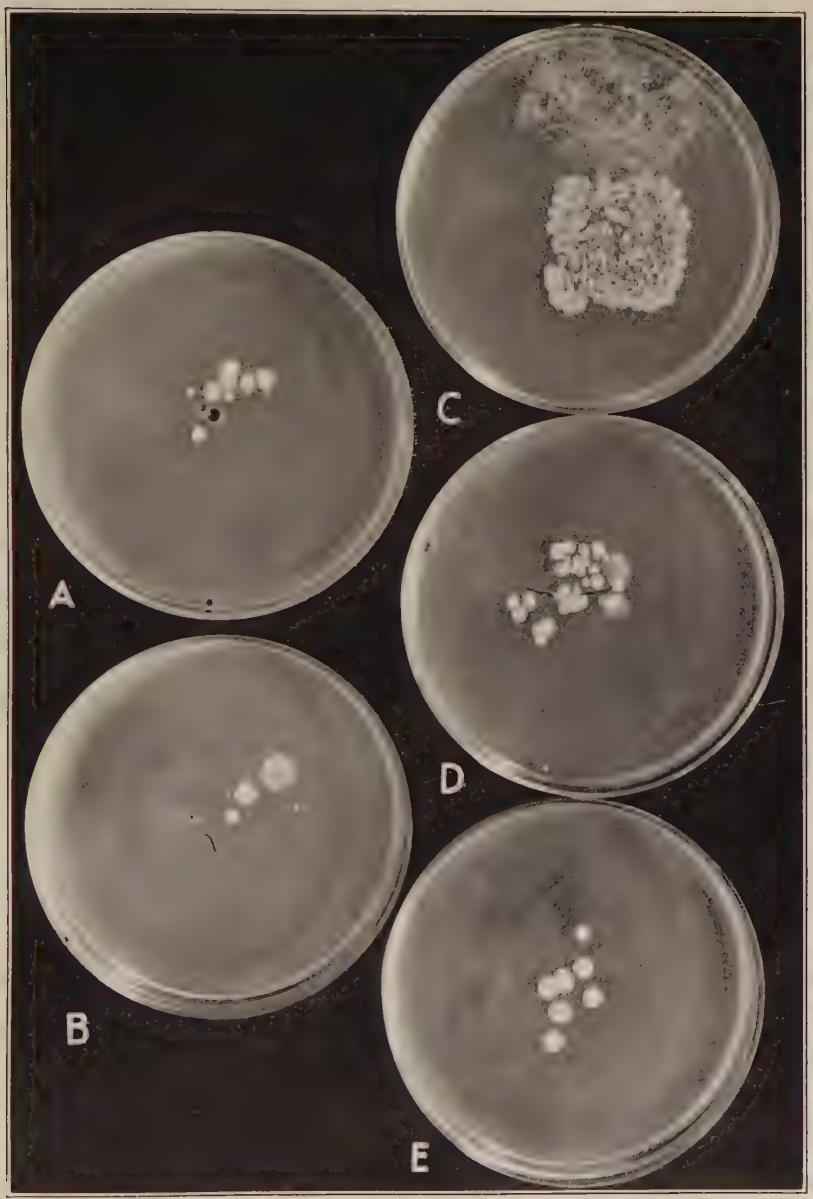
There is a general although irregular tendency for the killing effect to decrease for the later periods of exposure. It is probable that accidental screening of spores from the rays rather than wide difference in inherent protoplasmic resistivity accounts for the difficulty in killing absolutely all spores. The discontinuous applications average about the same as the continuous ones, when the third and succeeding applications are considered.

## DISINFECTION OF THE SURFACE OF ORANGES

The possible thoroughness with which the surface of oranges can actually be disinfected by the use of ultra-violet radiation was tested as follows: The fruits were dipped in a heavy suspension of *Penicillium digitatum* spores and dried; six hours later they were exposed for one-half, one, two, and four minutes to ultra-violet radiation at a distance of 13 inches; afterwards the previously radiated portion was gently rolled over very stiff nutrient agar solidified in Petri dishes, and these were incubated three days. From fruits exposed four minutes an average of 7 colonies per fruit developed; from those exposed two minutes, an average of 24 colonies; from those exposed one minute, an average of 21 colonies; from those exposed one-half minute, an average of 40 colonies; and from the unexposed checks, an average of 1,300 colonies. One series of plates from this test is shown in Plate 3.

Another method to test the sterility of the surface after irradiation was the following: The oranges were dipped in a suspension of *Penicillium digitatum* spores and dried; immediately afterwards they were exposed to the ultra-violet rays in five positions—two polar and three equatorial—each exposure being for 10 seconds; half the surface of each fruit was swabbed over with moist sterile cotton, the swabs were shaken up in 10 c. c. of sterile water, dilutions of 1/10 and 1/100 were made from this to nutrient agar, and plated. As a result of this procedure it was estimated that an average of 130,000 Penicillium spores were swabbed from half the surface of a nonirradiated fruit, and that the overlapping exposures of 10 seconds each on five different aspects of the fruit destroyed about 90 per cent of the spores.

In another similar test the oranges were divided into four lots. The fruits in one lot were given 6 overlapping exposures—2 polar and 4 equatorial—each exposure being for 30 seconds, at 13 inches from the lamp; in another lot the fruits were given 2 discontinuous exposures of 30 seconds each for each of the six aspects; in a third lot the fruits were given 3 discontinuous exposures of 30 seconds each; and the fourth lot was not exposed to the ultra-violet rays. By the swabbing method it was estimated that the fruit given a single set of 30-second exposures had about 400 Penicillium spores remaining viable; those given the double exposure, about 100 viable spores; those given the triple exposure, amounting to a total of 1½ minutes on each aspect of the fruit, about 10 viable spores; whereas the nonirradiated checks had about 40,000 viable spores. Expressed otherwise, the percentages killed by the three methods, based on these estimates, would be 99.000, 99.750, and 99.975, respectively. The more prolonged series of exposures, amounting to a total of 9 minutes for the fruit as a whole, killed approximately 3,999 out of 4,000 spores. It must be borne in mind that some of the few surviving spores might




Agar plates over the central portions of which oranges were rolled after having been dipped in a suspension of *Penicillium digitatum* spores, dried, and variously irradiated at a distance of 13 inches from the ultra-violet lamp. Photographed after incubation of the plates for three days: C, From an orange that was not irradiated; D, E, A, and B, from oranges irradiated for ½, 1, 2, and 4 minutes, respectively

be in advantageous positions for the starting of infection, such as in a wound crevice. One living spore so placed, under favoring conditions, might initiate rot just as certainly as though the fruit had not received treatment. There is no continuing retarding effect, as may occur from residues of a chemical disinfectant, and no protection from later contamination.

## HOLDING TESTS WITH ORANGES

Several practical tests of oranges were made to see how much reduction in development of actual rot could be brought about by exposing the surface of the fruit to ultra-violet radiations. In one of these tests the oranges (31 in each lot) were dipped in a suspension of *Penicillium digitatum* spores and were wounded by puncturing 100 times each. One lot was irradiated on six faces for one minute each at a distance of 13 inches from the lamp, another lot on six faces for one-half minute each, and a third check lot was not irradiated. At the end of three days the three lots had developed 13, 19, and 26 blue-mold rots, respectively; at the end of six days the totals for blue-mold rot were 31, 28, and 33, out of the 36 originally inoculated in each test. Swabbing tests of the surface of some of these fruits indicated an average of about 50, 48, and 1,000 viable spores per fruit for the three lots, respectively, which would mean about 95 per cent killing of the spores.

In another test, two lots of 32 fruits each were inoculated and punctured as described above. One lot was exposed to ultra-violet radiation for 10 seconds each on five faces at a distance of 9 inches from the lamp, while the other lot was not treated with the rays. After six days holding at room temperature the blue-mold rots were 14 and 13, respectively; and after nine days they were 26 and 30, respectively, out of the 32 fruits originally in the test.

In still another test, 200 fruits were inoculated and wounded. Half of them were irradiated for 10 seconds on each of six faces at a distance of 13 inches from the lamp, and half were not irradiated. The progressive development of total blue-mold rot is indicated as follows: Third day, 9 per cent for the treated and 34 per cent for the untreated; sixth day, 62 and 86 per cent; ninth day, 78 and 92 per cent; twelfth day, 83 and 93 per cent; fifteenth day, 85 and 94 per cent.

No appreciable effect on the development of stem-end rot was noted in any of the tests. In two instances special tests for the effect in checking stem-end rot gave negative results. There was no evidence of any deleterious effects to the fruit in any of the tests as a result of the exposures to ultra-violet rays.

While there is some evidence that the ultra-violet treatment retards the development of rot during the first few days of holding, the final amounts of rot during a usual marketing period are very much the same in the treated and untreated lots in all tests. The conditions of these tests were purposely made very severe in order to give every fruit a chance to become infected except as the ultra-violet treatment might prevent such infection. In commercial practice only a small percentage of the fruit ordinarily would be exposed to such severe infection. These fruits, however, are the ones that require protection, which should be adequate to insure their preservation under very severe conditions.

## RELATIVE FUNGICIDAL ACTION OF DIFFERENT WAVE LENGTHS OF LIGHT

It has been seen that, taking the ultra-violet radiation as a whole, the fungicidal effect varies with the time of exposure and with the intensity of the light. The latter varies inversely as the square of the distance from the source and may be affected to some degree by imperfect transmission through media intervening between the source and the fungus material to be acted upon. In the investigation of bactericidal action previously referred to, the writers have tested the spectral range of abiotic action on *Bacillus coli* by using mica or glass screens that transmit the different regions of the ultra-violet spectrum in different degrees. It was found that germicidal action for this organism is produced by ultra-violet radiation extending from the shortest wave lengths tested (about 240 $\mu$) to and including 365 $\mu$. The action probably extends beyond this upper limit, but in very weak degree. The shortest rays have the most violent lethal action, which decreases in intensity with increase in wave length. By the same method of using mica or glass screens, preliminary tests with fungus spores bear out the same general conclusion, namely, that different wave-length components have different killing power, which increases as wave length decreases over a wide range, the lethal action beginning somewhere about 365 $\mu$ as an upper limit.

As a result of absorption in the atmosphere, sunlight at the earth's surface has an ultra-violet component with wave length extending to about 313 $\mu$ as the lower limit. This corresponds to the upper and less-effective range of ultra-violet radiation from a mercury-arc lamp. Consequently exposures to sunlight to be lethal for fungi must be much more prolonged than in the case of radiation, as from the mercury arc, which includes quick-killing shorter wave lengths down to 240 $\mu$. Ordinary glass intercepts some of the shorter wave-length rays reaching the earth in sunlight and reduces its fungicidal action. A test was made by exposing spores of *Penicillium digitatum* and of *Phomopsis citri* for 16 minutes to radiation from a glass-inclosed mercury-arc lamp. No killing effect could be determined. Furthermore, the intervention of ordinary thin microscope slides during exposure to emanations from the quartz-mercury lamp afforded complete protection for periods of exposure that produced a high percentage of killing when the light was unobstructed.

## CONCLUSIONS

Ultra-violet rays are capable of killing fungus spores, in most cases after a comparatively brief exposure. Some types of spores require very much longer exposures than others for killing, probably on account of the difficulty in ray penetration of the spore walls due to their protective coloration or to their composition. The outer spores of clumps have a protective shading effect on those in the center of the mass, and the latter survive exposures several times longer than is requisite to kill the single detached spores. Growing mycelium is more easily killed than are ungerminated spores. Mycelium may penetrate a substance that is opaque to the rays and thus escape the killing effect.

The fungicidal effectiveness of ultra-violet treatment depends on the intensity of the radiation and on the wave lengths of the component rays. The shorter the wave length the more lethal the effect with a given intensity. The killing effect is cumulative, several short intermittent exposures having almost the effect of one continuous exposure of the same total time. The ultra-violet rays, especially those of shorter length, have a very limited penetrating power. The yellow or orange coloration of mature citrus fruits acts as a very effective protective screen to prevent penetration by the rays. Irregularities in the fruit surface or the presence of foreign particles would tend to have a shading effect on some of the spores.

The spores that are in the best position actually to start decay are the ones that are in punctures or other breaks in the skin, and these are most likely to be protected from full exposure to the rays. Mycelium that has penetrated the citrus tissue to any extent could not be reached by the rays. The ultra-violet treatment does not afford protection from later contamination with blue-mold spores. In addition to these limitations there is the difficulty and expense of making a mechanical installation that will insure complete and sufficiently prolonged exposure of all parts of the fruit under packing-house conditions. On the whole it would seem that other methods of treating citrus fruit to prevent blue-mold rot are more feasible and economical at the present time than the use of ultra-violet radiation as it is now capable of being produced and applied commercially.

Ultra-violet radiation has been successfully used for the disinfection of water. Its limitations here are the expense and the comparatively small volume of water that can be treated in a given time. It is possible that the wash water used in citrus packing houses might be disinfected by this means, but under ordinary circumstances it would be less expensive to renew the wash water than to incur the relatively high cost of using ultra-violet radiation for the purpose of keeping it sterile.

## SUMMARY

Spores of 27 miscellaneous species of fungi were exposed for one minute to ultra-violet radiations from a quartz-inclosed mercury-tungsten arc at a distance of 6 inches. There was complete killing of the spores of 16 species, a survival of less than 1 per cent in the case of 4 species, and survival ranging from 2 to 53 per cent for the remaining 7 species. Mycelium from germinated spores was more easily killed than the resting spores.

The killing effect of ultra-violet rays is increased with the lengthening of the time of action and also with the increase in the intensity of the radiation. Decrease in the distance from the source of radiation results in increase in intensity and in consequent killing effect. An exposure of 5 seconds at 6 inches was sufficient to kill at the rate of 907 out of 1,000 spores of *Penicillium digitatum*. With an exposure of 45 seconds the rate of killing was 998 out of 1,000. It is supposed that partial shading of some of the spores accounts for the accidental survival of a few.

A series of short exposures has almost the same effect in a cumulative way as one continuous exposure of equal total duration. Penicillium spores on the surface of citrus fruits can be killed in large proportions by intermittent short exposures to ultra-violet rays.

The ultra-violet rays in the dosages used did not cause any apparent injurious effect on the oranges.

In holding tests, decay of inoculated oranges was only moderately retarded by applications of ultra-violet rays that seem to reach the limit of economical practice under present conditions for time of exposure and distance from lamps.

The principal physical limitations to the use of ultra-violet rays for complete disinfection of fruits seem to be their inability to penetrate much if any below the surface, so as to destroy mycelium that may have started infection already, and the accidental shading effects that favor the survival of some of the contaminating spores.

The shorter wave-length components of the ultra-violet spectrum have the greatest fungicidal action. The longer wave lengths of this spectrum, such as are components of sunlight, have relatively weak fungicidal power, and require greatly prolonged exposures for the killing effect.

○